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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

APR 7 - 1987

pc 032001

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Peer Review on 2,4-D

FROM: Reto Engler, Chief
Scientific Mission Support Staff
Toxicology Branch/HED (TS-769)

TO: Addressees

Attached is a comprehensive package on 2,4-D prepared by Dr. M. Van Gemert for your review. In Appendix F you will also find an evaluation of epidemiological studies by Jerome Blondell.

A meeting to discuss and evaluate the weight-of-the-evidence on 2,4-D has been scheduled for Thursday, April 23, 1987, at 1:00 to 3:00 PM in Room 1119 of CM-2.

Attachment

ADDRESSEES:

T. Farber
W. Burnam
J. Quest
E. Rinde
R. Levy
L. Kasza
J. Hauswirth
J. Blondell
D. Anderson
H. Spencer
A. Barton
R. Beliles
M. Van Gemert
D. Beal
D. Barnes/R. Hill

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

Subject: The Weight of the Evidence Evaluation for the Oncogenic Potential of 2,4-dichlorophenoxy acetic acid (2,4-D)

From: Marcia van Gemert, Ph.D.
Head, Section III
Toxicology Branch, HED
M. van Gemert 3/31/87

Thru: Theodore M. Farber, Ph.D.
Chief, Toxicology Branch, HED
Theodore M. Farber 3/31/87

Attached is a report prepared for the Peer Review Committee on 2,4-D. Data are provided so that a "Weight of the Evidence" determination may be made regarding the oncogenic potential of 2,4-D.

Attachment

A set of scientific issues being considered by the Agency in connection with 2,4-Dichlorophenoxyacetic acid.

Introduction:
2,4-Dichlorophenoxyacetic acid (2,4-D) is a growth regulator and herbicide on broad-leaf plants and has been used extensively for 40 years. It is a phenoxyacetic acid herbicide with the following chemical structure.

The relevant oncogenicity base consists of one rat chronic/oncogenicity study, one mouse oncogenicity study, and a population-based case-control epidemiology study on farm workers from the state of Kansas. The rat study has shown an increased incidence of astrocytomas at the high dose, and the epidemiology study had identified an association between increased incidence of non-Hodgkin's lymphoma and phenoxyacetic acid herbicides, particularly 2,4-D. The Agency requests that the Panel consider our assessment of the weight of the evidence as presented below.

Weight of Evidence:
2,4-D was administered to CDF (F344/Crl-Br) rats at doses of 0, 1, 5, 15, and 45 mg/kg/day for 2 years with an interim sacrifice at 53 weeks. The increases in astrocytomas produced by the two highest dose levels of 2,4-D in treated male rats (i.e., 2/58 or 3.4% at 15 mg/kg/day; and 6/60 or 10% at 45 mg/kg/day) were not statistically significantly elevated *per se* when compared to control male rats (i.e., 1/60 or 1.6%) by the Fisher Exact test. The Cochran-Armitage trend test was highly significant for astrocytomas in males, $P < 0.00005$. The increased incidences of astrocytomas observed in the treated animals (and also that observed in the controls) exceeded the historical control incidence of astrocytomas ($0.4 \pm 1.0\%$) in recent studies conducted by the NTP. See Table I below for tumor incidence.

Table I
INCIDENCE OF ASTROCYTOMAS

	Males					Females				
Group mg/kg	0	1	5	15	45	0	1	5	15	45
Unscheduled Deaths	1/18	0/7	0/3	2/7	1/14	0/10	1/13	0/13	0/12	0/14
52-week Sacrifice	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
104/105-week Sacrifice	0/32	0/43	0/47	0/41	5/36	0/40	0/37	1/37*	1/38	1/36
All Animals	1/60	0/60	0/60	2/58	6/60†	0/60	1/60	1/60	1/60	1/60

† Significantly different from controls, $P < 0.054$, using Fishers Exact Test; using Cochran-Armitage Trend Test, $P < 0.00005$
* A second animal was diagnosed as having an astrocytoma according to the study text. However, this finding was not confirmed by Dr. James Swenberg in his review of these slides.

The Peer Review Committee concluded that an MTD may not have been reached for the male rats at the high dose (45 mg/kg/day). The toxicological changes produced in female rats at 45 mg/kg/day are somewhat more marked, and suggest that 45 mg/kg/day might be closer to an MTD. The Committee recommended that a repeat, modified oncogenicity study of 2,4-D be performed in F344 male and female rats using greater numbers of animals and higher doses of 2,4-D than previously employed. However, only the brain (numerous sections) should be examined for tumors.

2,4-D was administered in the diet to $B_6C_3F_1$ Crl Br mice for 24 months, The doses fed were 0, 1, 15 and 45 mg/kg/day and produced no observed oncogenic response (data not presented in this summary but available in the Peer Review package, #4). The Peer Review Committee concluded that an MTD had not been reached in this study and recommended that the study be repeated.

The epidemiology study that was considered by the Peer Review Committee was titled "Agricultural Herbicide Use and Risk of Lymphoma and Soft Tissue Sarcoma, by S. Hoar, A. Blair, F. Holms, et al., JAMA, 256: 1141-1147, 1986. This was the report of a population-based case-control study conducted by the National Cancer Institute on farm workers in Kansas. The report stated that they had found an association between farm herbicide use (phenoxyacetic acids) and non-Hodgkin's lymphoma, but did not find an association with soft tissue sarcoma or Hodgkin's disease. More detailed analysis suggested that the non-Hodgkin's lymphoma association was most likely due to chlorophenoxy herbicides, especially 2,4-D. The Peer Review Committee questioned the accuracy of the exposure categorization among the study subjects and other factors were identified which might plausibly account for the increased risk of non-Hodgkin's lymphoma. The Committee deferred a carcinogenic weight of the evidence classification of

the epidemiologic data using EPA Guidelines pending the receipt of further human data involving 2,4-D in the near future. The National Cancer Institute has two studies underway which should help determine whether the 2,4-D/non-Hodgkin's lymphoma association can be substantiated.

According to the WHO report of 1984 on 2,4-D, the available mutagenicity studies are inadequate to evaluate the genetic effects of 2,4-D and its derivatives in short-term tests.

Two other structurally related phenoxyacetic acids, 2,4,5-T (trichlorophenoxyacetic acid) and Silvex (2,4,5-trichlorophenoxy propionic acid) were tested chronically and found not to be carcinogenic. However, the dioxin contaminent TCDD (2,3,7,8-trichlorodibenzo-p-dioxin), found in both 2,4,5-T and Silvex, has been found to be carcinogenic. A third phenoxyherbicide, 2,4-DP (2,4-dichlorophenoxy-2-propionic acid) was tested in both rats and mice and found to be carcinogenic in rats. This determination, however, is presently being re-examined.

Based on the weight of the evidence, the Peer Review Committee concluded that the data available for 2,4-D provide only limited evidence of oncogenicity for the chemical in male rats. According to the EPA Guidelines for Carcinogen Risk Assessment the Committee classified 2,4-D as a Interim Category C oncogen (possible human carcinogen with limited evidence of carcinogenicity in animals). That is, 2,4-D produced benign (although life-threatening) tumors of marginal statistical significance in one species of one animal in a single study where the MTD may not have been reached in males. No compound-related tumors were observed in mice. In addition, mutagenicity data and structure-activity relationship information provided weak and relatively unconvincing support for the oncogenicity of 2,4-D. Epidemiology data on phenoxy acetic acid herbicides and non-Hodgkin's lymphomas in farmers did not provide a definitive link between the use of 2,4-D per se and human oncogenesis. The interim category C classification was assigned to 2,4-D pending the receipt of two additional oncogenicity studies in rodents (rats and mice) and additional epidemiology data in humans.

Issue: Does the Panel agree with the Peer Review Committee's conclusion concerning the Interim Category C classification of the available 2,4-D oncogenicity data?

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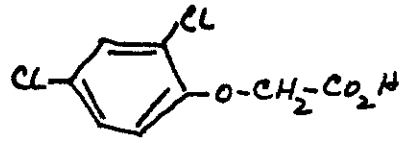
2. critical review of NCI study by Jerome Blondell, EPA
3. Review by Martha S. Linet, MD., MPH
4. Review by Leon F. Burmeister, Ph.D., U. of Iowa
5. Review by Donald P. Morgan, MD Ph.D., U. of Iowa
6. Review by Brian Macmahon, MD Ph.D.

G. Toxicology Branch "one-liners"

REPORT FOR THE PEER REVIEW COMMITTEE FOR ASSESSING
THE ONCOGENICITY OF 2,4-Dichlorophenoxy acetic acid

I. Summary:

The Industry Task Force for 2,4-D Research Data has submitted data in response to a data call in for 2,4-D. 2,4-D has been used extensively as a growth regulator and herbicide on broad-leaf plants for 40 years. A large WHO monograph was printed in 1984 summarizing all the available literature to that data and is appended for reference in Appendix A. The chemical structure is:



2,4-D contains some potentially ~~hazardous dioxin contaminants~~ including the di, tri and tetra chlorodibenzodioxins.

2,4-D is not well absorbed through skin, however, is fairly well absorbed orally and the volume of distribution is 20-50% of body mass as volume. 2,4-D does not appear to be significantly metabolized, however, the metabolite 2,4-dichlorophenol (2,4-DCP) can be found as a residue in ruminants, probably due to bacterial degradation of 2,4-D in the rumen. 2,4-D conjugates have been found in urine of several species, including man. 2,4-D is mainly excreted in urine, and to a lesser extent in feces. Half-life in humans from a single exposure can be from 24 to 48 hours.

2,4-D is structurally related to 2,4-DB, MCPA, Mecoprop, 2,4,5-T, fenoprop and 2,4,5-TP. 2,4,5-T and fenoprop both contain the carcinogenic dioxin TCDD. However, when both herbicides were tested in carcinogenesis bioassays with the TCDD contaminant present, the results were negative. 2,4,5-TP was found to be carcinogenic in rats but not mice.

Subchronic and chronic effects include vomiting, diarrhea, muscle weakness, muscle spasms (myotonia), reduced food and water consumption, weight loss, CNS depression, damage to myocardium, various hematological and blood chemistry changes, hepato- and nephrotoxicity and endocrine organ toxicity.

In the teratology study submitted by the Industry Task force there was a slight delayed ossification seen with no maternal effects seen. In a submitted 2-generation study in rats, nephro-toxicity was seen in F_p and F_1 male parents with reduced pup weights seen in F_1b pups.

According to the recent (1984) WHO report the present available mutagenicity studies are inadequate to evaluate the genetic effects

effects of 2,4-D and its derivatives in short-term tests.

2,4-D was tested in the $B_6C_3F_1$ mouse for 24 months at doses of 0, 1, 15 and 45 mg/kg, and found not to increase the incidence of tumors. It was, however, nephrotoxic causing increased cytoplasmic homogeneity of the renal tubular epithelium due to a reduction of cytoplasmic vacuoles. Adrenal and kidney weights were effected by treatment.

A 2-year combined chronic/oncogenicity study in rats at doses of 0, 1, 5, 15, and 45 mg/kg/day revealed an increased incidence of a rare brain tumor, astrocytoma, in high dose males. The incidence in males was 1/60, 0/60, 0/60, 2/58, 6/60 for males and 0/60, 1/60, 1/60, 1/60, 1/60 for females in groups 1-5 respectively. Other effects seen included increased kidney weights, increased brown tubular pigment in kidneys from groups 3, 4, and 5, increased incidence of pelvic microcalculi in group 4 and 5 males and group 5 females. There was an increase in transitional epithelial hyperplasia of the kidney in group 5 females.

A population-based case control epidemiology study conducted by the NCI was done in Kansas where an association was found between herbicide use and non-hodgkins lymphoma. Other epidemiology studies have recently been completed and are in the process of being reviewed by Jerome Blondell and are considered negative by Mr. Blondell for an association between 2,4-D and non-hodgkins lymphoma or other tumors.

II. Contaminants:

Several potentially hazardous contaminants have been identified and recently more sensitive and specific methods have become available to quantitate them. These contaminants include the di-, tri-, and tetrachlorodibenzo-p-dioxin isomers (structures given below) and N-nitrosamines. The most toxic CDD, namely 2,3,7,8-TCDD, is not normally found in 2,4-D products. However, there have been some cases where manufacturing equipment was used to produce both 2,4,5-T and 2,4-D resulting in cross contamination of 2,4-D with 2,4,5-T and 2,3,7,8-TCDD. Except for the NCI studies on 2,7-dichlorodibenzo p-dioxin (which was reported to be negative in rat and negative in female, equivocal in male mouse), the chronic toxicity of the CDDs found in 2,4-D has not been tested.

Contaminants are on next page

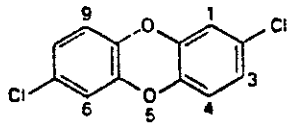
III. Metabolism:

This is a brief summary of the WHO text, pages 54-62 and the full WHO report is appended in appendix A for more detailed examination.

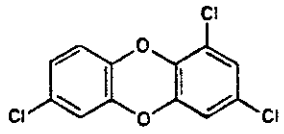
Absorption:

Inhalation: 2,4-D appears to be rapidly absorbed by inhalation, following first order kinetics with an absorption half time of 1.4 to 1.7 minutes in rats.

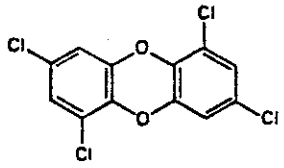
Pg 2A



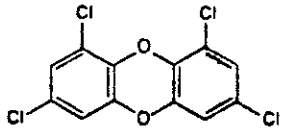
2, 7 – dichlorodibenzo-p-dioxin



1, 3, 7 – trichlorodibenzo-p-dioxin



1, 3, 6, 8 – tetrachlorodibenzo-p-dioxin



1, 3, 7, 9 – tetrachlorodibenzo-p-dioxin

WHO 83982

Fig. 2. Polychlorinated dibenzo-p-dioxin (CDD) byproducts.

CONTAMINANTS FOUND IN 2,4-D -
THESE CDDs HAVE NOT BEEN TESTED FOR CARCINOGENICITY

Dermal: Some work has been done to indicate little absorption occurrs through skin. One human volunteer study indicated that only 5.8% of the administered dermal dose of ^{14}C-labeled 2,4-D was excreted in urine. Occupational exposures have suggested a fairly efficient dermal exposure, however, surfactant properties of solvents have not been adequately evaluated concerning dermal penetration.

Oral: In human volunteers there was individual variation in rate and extent of absorption from the GI tract, however, absorption is fairly rapid and 2,4-D is completely absorbed from the human GI tract, and follows first order kinetics.

Distribution: There appears to be more than one physiological compartment for 2,4-D storage. The volumes of distribution appear to be 20-50% of the body mass in volume. 2,4-D is reversibly bound to plasma proteins, particularly albumins, and compete for binding sites with related compounds. Approximately 17% of an administered dose crosses the placenta in mammals.

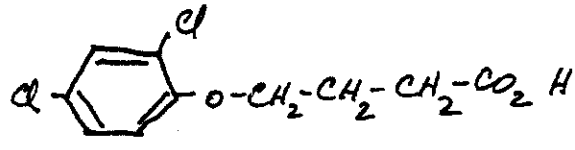
Metabolism: Rats and pigs appear to hydrolyse 2,4-D esters both in the gut and after absorption in the body. However, this finding is open to question. 2,4-D does not appear to be significantly metabolized in animals except for ruminants. The metabolite 2,4-dichlorophenol (2,4-DCP) has been found as a residue in ruminants and is probably there due to bacterial degradation of 2,4-D in the rumen. 2,4-D appears to form conjugates in the kidney tubules. Taurine and glycine conjugates have been found in urine of rats, pigs, chickens and the dogfish shark. In human urine conjugates have been found at up to 27% of the ingested dose.

Tissue residues: Highest tissue residue levels have been found in liver, kidney, lungs, spleen and heart in several mammals studied. However, there is no evidence for any significant bioaccumulation of 2,4-D or conjugates.

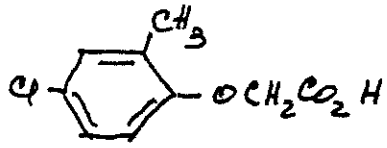
Elimination: 2,4-D is mainly excreted in urine and to a lesser extent in bile and feces. The half-life in human volunteers is about 24 hours, depending on circumstances, although estimates from single industrial exposures place the half-life between 35-48 hours.

IV. Structure-activity relationships

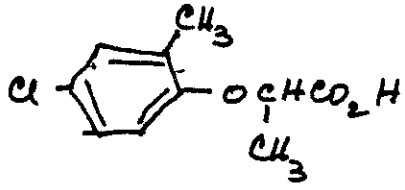
There are six herbicides that are structurally related to 2,4-D.



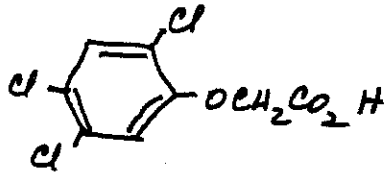
1. 2,4-DB - 2,4 dichlorophenoxy butyric acid: Has not been tested chronically.



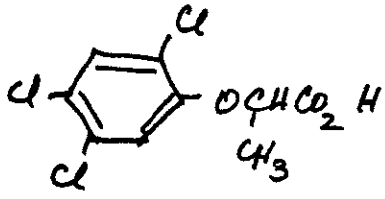
2. MCPA - (4-chloro-2-methylphenoxy acetic acid) has not been tested chronically



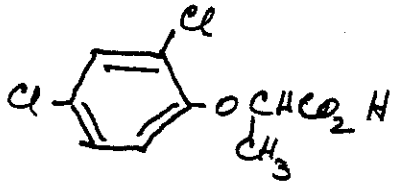
3. MCPP, Mecoprop, (2,4-chloro-2-methylphenoxy propionic acid) has not been tested chronically



4. 2,4,5-T, (trichlorophenoxyacetic acid) was found not to be carcinogenic in rat and mouse. The contaminant TCDD was found by CAG to be a more potent carcinogen than aflatoxin, producing hepatocellular hyperplastic nodules and hepatocellular carcinomas. TCDD produced fetotoxicity and embryolethality and reproductive effects. 2,4,5-T mutagenicity was done by NIEHS according to Hank Spencer, and the results were negative. 2,4,5-T was not cancelled for all uses. There is still a registered use for rangeland, and vines in orange orchards.



5. Fenoprop, Sylvex (2,4,5-trichlorophenoxy propionic acid) is fetotoxic and teratogenic in rat but not carcinogenic in rat. Silvex contains the same TCDD contaminant as 2,4,5-T. Not all uses were cancelled. It is still used for pear ripening.



6. 2,4-DP, (2,4-dichlorophenoxy-2-propionic acid) was tested in
   A. Sprague Dawley rat and found:
      1. increased incidence and frequency in males by dosage of malignant tumor types compared to controls.
      2. increased incidence in males and frequency of three specific tumor types: pituitary, thyroid and brain carcinomas.
      3. A decrease in life span in male rats with pituitary and brain tumors.
      4. a shift with dose in the malignant tumor pattern in male controls to the malignant tumor pattern in the male treated groups. The treated groups had 85-86% of pituitary and thyroid malignant tumors whereas the controls had 37% of these tumor types.
      5. Increased tumor load with dose (number of tumors/rat) in male rats.
   B. 18-month oncogenicity study in mice was negative for oncogenicity
   C. Chronic/oncogenicity study in Fisher-F344 rats was negative for oncogenicity
   D. Teratology study in rabbits- causes developmental toxicity
      Teratology study in rats was negative.
   E. Reproduction study in rats- causes increased mortality during lactation.
   F. Mutagenicity: was found to be mutagenic in several tests when tested without S-9 activation.

V. Non-oncogenic effects:

Acute effects:

Skin and eye irritation: According to the WHO report 2,4-D does not appear to be an eye or skin irritant.

Skin sensitization: Adequate information on the skin sensitization potential of 2,4-D is not available.

LD_{50}:

Species	Sex	LD_{50} (mg/kg B.W.)
mouse	m	375
mouse	m	368
rat	m	375
rat		666
guinea pig	m + f	469
guinea pig		1000
rabbit		800
dog		100
chicken	m + f	541

Acute dermal: Several literature reports indicate that under extreme test conditions mice and rats may absorb lethal amounts of 2,4-D amine salts and esters through skin. However, no acute dermal LD_{50} studies are reported on 2,4-D in the literature.

Acute inhalation: No reports are available

Subchronic and chronic effects: (summarized from the WHO report)

1. Observations of toxicity: These include diarrhea, ~~vomiting~~ ~~dysphagia~~, decreased gut motility, irritation and necrotic changes. Characteristic signs of severe 2,4-D poisoning in mammals include muscular weakness, stiffness, stilted gate and muscle spasms (myotonia) especially in hind limbs of rodents. Muscular incoordination can progress to paralysis. At high doses 2,4-D may act as a central nervous system depressant, causing lethargy slowed respiration, stupor, coma and death.

2. Effects on food and water consumption: High concentrations of 2,4-D or derivatives may cause a reduction in food and water consumption and weight loss or reduced body weight gain in rats dogs, pigs, cattle, sheep, and rabbits.

3. Effects on CNS: 2,4-D is a central nervous system depressant at high concentrations and appears to be related to a partial breakdown of the blood-brain barrier, possibly as a result of damage to capillary vessels and a subsequent accumulation in the CNS.

4. Effects on the peripheral nervous system: More studies are needed on this subject. 2,4-D does not appear to cause peripheral neuropathy. The partial or ~~complete paralysis~~ seen in 2,4-D ~~intoxication especially~~ in hind limbs of rats may be a myotoxic rather than a neurotoxic mechanism.

5. Cardiovascular effects: 2,4-D at high doses may cause biochemical ~~physiological~~ and structural damage to the myocardium ~~in vitro~~ and in vivo, as part of its myotoxic action.

there seems

6. Hematologic effects: According to the WHO report, shifts have been reported in the number or types of erythrocytes, leukocytes bone marrow cells or changes in hemoglobin levels in a variety of laboratory and domestic animals from 2,4-D ingestion.

7. Blood chemistry: Some investigators have found 2,4-D administration changes various serum, plasma or erythrocyte enzyme activity levels, and electrolytes. Blood proteins and glucose as well as SGOT, SGPT and BUN changes may be secondary to myotoxic nephrotoxic or hepatotoxic effects seen with 2,4-D.

8. Nephrotoxicity: Many studies in the literature and in the submitted studies indicate that the kidney is a target organ for the structural, physiological and chemical effects of 2,4-D. Signs of toxicity at high doses include impaired kidney function, increased relative kidney weight and gross and histological

abnormalities (parenchymatous degeneration, hypertrophy and hyperplasia, cloudy swelling especially in the cells of the proximal convoluted tubules and glomerular lesions). Additional more detailed descriptions of 2,4-D induced nephrotoxicity are found in section IX in the 2 life-time rat and mouse studies submitted by the Industrial Task Force for 2,4-D Research Data.

9. Hepatotoxicity: After prolonged treatment with toxic doses of 2,4-D subacute toxic hepatitis is seen with congestion of hepatic blood vessels, and cloudy swelling, fatty infiltration, focal necrosis, degeneration or atrophy of hepatocytes, especially of the parenchyma in the centrolobular areas. High doses of 2,4-D have also been reported to induce peroxisome proliferation and increased levels of mixed function oxidases in liver cells of rats and hamsters.

10. Endocrine organ toxicity: Swelling and congestion of the thyroid were noted in cattle and sheep after fatal poisoning with various 2,4-D products. 2,4-D also appears to effect adrenal function.

VI. Reproductive and developmental studies:

The scientific literature provided studies of little value for regulatory purposes on this subject.

Submitted studies:

1. Rat teratology study dated 3/2/83 from Wil laboratories. Pregnant Fisher 344 rats were given 0, 8, 25, or 75 mg/kg of 2,4-D between days 6-15. No maternal effects were seen and at the high dose there was a slight increase in delayed ossification.

2. 2-generation reproduction study in rats dated 9/30/86 from Will research labs tested at doses of 0, 5, 20, or 80 mg/kg/day. Results indicated renal tubular degeneration of the males of the F_0 and F_1 generations. Cortical tubular degeneration (observed mostly in the proximal convoluted tubules) was confined to f_0 males. There was reduced pup weight in the F_1b pups.

VII. Mutagenicity:

According to the WHO report, the available studies are inadequate to evaluate the genetic effects of 2,4-D and its derivatives in short-term tests.

do mouse first

VII. Summary of lifetime studies submitted by the Industrial Task Force for 2,4-D Research Data.

1. 24-month rat combined chronic/oncogenicity study

Performing laboratory: Hazleton Laboratories, Vienna Virginia

Date: May 29, 1986.

2,4-D technical (97.5% purity) was administered to CDF (F 344/CRL-BR) rats at doses of 0, 1, 5, 15, and 45 mg/kg/day for two years. Each group contained 60/sex/dose with an interim sacrifice of 10/sex/dose at 53 weeks. Hematology, clinical chemistry and urinalysis were collected from 10 rats/sex/group at initiation and following weeks 26, 52 and 78. Clinical chemistry analyses were also performed on all animals surviving to termination of the study.

There was no treatment-related effect on survival. Male mortality at 24 months was 18, 7, 2, 8 and 12, and female mortality was 10, 13, 2, 8 and 12 for groups 1-5 respectively. Females of group 5 showed a statistically significant decrease in body weight gain for weeks 0-52 and 0-104 with an accompanying decrease in food consumption for weeks 0-52. There was a slight increase in albumin and a slight decrease in globulin at week 105 in group 5 males, increasing the AG ratio at weeks 79 and 105. There was an increase in serum alanine aminotransferase in males and females at week 105 in group 5. T_4 was slightly depressed at 105 weeks in group 5 females.

weights

At 52 weeks, all kidney weight parameters measured for group 5 males were elevated along with absolute and kidney/body weight ratios for females of group 5. At terminal sacrifice group 5 females had increased kidney weight parameters and group 4 females also had increased kidney/body weight ratios. Male kidney weight ratios were elevated, but not significantly. There was a dose-related increase at 104 weeks in all male thyroid/parathyroid parameters with statistical significance in group 4 males and females and group 5 male thyroid/body weight ratios. Organ/body weight changes were seen in group 5 females for ovaries and brain stem and all female group 3 and 5 pituitary weight parameters were elevated.

At the 52 week sacrifice histopathologic examination revealed that there were general alterations in histopathological parameters in the kidneys of groups 3,4 and 5 that appeared compound-related. These consisted of:

1. an increased incidence in brown tubular cell pigment in the males of groups 3, 4, and 5 (9/10, 10/10, 10/10 respectively) and groups 3, 4, and 5 females (5/10, 6/10, and 7/10 respectively) when compared to control males (2/10) and control females (3/10).
2. An increased frequency and severity of fine vacuolization of cytoplasm in the renal cortex in group 5 females (8/10) when compared to control females (5/10) and an increase in severity in groups 3 and 4 females when compared with control females.

this was not seen in final sacrifice

At the terminal sacrifice compound-related histopathologic alterations occurred in the kidneys of groups 3, 4, and 5 males and females. These included:
1. increased brown tubular cell pigment in the kidneys of groups 3, 4, and 5 males (8/47, 18/41**, and 18/36** respectively) and groups 3, 4, and 5 females (23/37*, 19/38**, 13/36 respectively) when compared to control males (2/32) and females (8/40).
2. Increased incidence of pelvic microcalculi in groups 4 and 5 males (8/41, 9/36 respectively) and group 5 females (28/36**) when compared to control males (2/32) and females (19/40).
3. A slight increase in frequency of transitional epithelial hyperplasia in group 5 females (6/36) when compared to controls (0/40).
Additional histopathology tables were requested and the submitted information concerning granulomatous prostatitis, kidney transitional cell hyperplasia and microcalculi are discussed in more detail on page 2 of appendix D.3.

Increase in groups 4+5 associated w microcalculi

The most significant finding in this study involved a rare, small brain tumor-astrocytoma (glioma) found in high dose males. In the original report the study text had stated that the tumors seen for the groups was:

Incidence of Astrocytomas as given in study text

	MALES					FEMALES				
group	1	2	3	4	5	1	2	3	4	5
mg/kg/day 2,4-D	0	1	5	15	45	0	1	5	15	45
Unscheduled deaths	1/18	0/7	0/3	2/7	1/14	0/10	1/13	0/13	0/12	0/14
52-week sac.	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
104 wk sac.	0/32	0/43	0/47	0/41	5/36	0/40	0/37	2/37	1/38	1/36
All animals	1/60	0/60	0/60	2/58	6/60	0/60	1/60	2/60	1/60	1/60

Task force set -

Dr. Koestner of Michigan State re-reviewed the brain slides for the Industry Task Force (appendix D.2). He differed from the original pathology report on only one point. He felt that the high dose male animal # 23473, originally diagnosed as having an astrocytoma, actually had a mixed glial and mesenchymal cell population. He presented arguments concerning the biological criteria for evaluation of neurocarcinogens, summarized on page 2 of appendix D.2 and gave historical control incidences for glioma summarized on page three of this appendix. These historical controls are summarized below.

NTP data base Should be one

Historical Controls

VARIABILITY IN BRAIN GLIOMA INCIDENCE IN CONTROL MALE SPRAGUE-DAWLEY RATS 1 YR. AND OLDER (SELECTED FROM SWENBERG, J.A. 1986)

NUMBER	COLOR	LABORATORY	CONTROL 1(%)	CONTROL 2(%)	CONTROL 3(%)
1-5	Diff. Colors	IRDC	0/292 (0)	2/287 (0.7)*	2/137 (1.4)
6	Red No. 33	IRDC	3/57 (5.2)	0/59 (0)	2/58 (3.4)
7	Green No. 3	Biodynamics	0/52 (0)	5/55 (9)**	--
8	Blue No. 2	Biodynamics	0/59 (0)***	2/59 (3.4)	--
9-13	Diff. Colors	Biodynamics	2/290 (0.7)	2/289 (0.7)	4/231 (1.7)
14	Red No. 9	Litton	4/58 (6.9)****	6/60 (10)	2/57 (3.5)
15	Red No. 27	Litton	2/54 (3.7)	0/55 (0)	--
16	Red No. 36	Litton	2/57 (3.5)	1/59 (1.7)	0/53 (0)
17	Red No. 30	Hazleton	3/59 (5.1)	1/55 (1.8)	--

7/55 according to FDA counting and SQUIRE Counting

* One of the rats died on day 350 with a glioma
** Additional sections resulted in 6/55 (10.9%)
*** Additional sections resulted in 2/59 (3.4)%
**** One glioma diagnosed at 12 mos. interim sacrifice

RANGE of HISTORICAL CONTROLS = 0-10% *

Dr. James Swenberg of CIIT was asked by EPA to re-evaluate the brain slides originally diagnosed as having astrocytomas. For all but one animal, he agreed with the original study text diagnosis of astrocytomas. He disagreed with the astrocytoma diagnosis of female B23289 in the 5 mg/kg dose group. He diagnosed this lesion as a focal area of gliosis present near the center of the olfactory bulb with no neoplasm detected. The original study text had diagnosed this area as an astrocytoma. Dr. Swenberg also disagreed with Dr. Koestner's diagnosis of the high dose male B 23473. Dr. Koestner had diagnosed this male as having an area in the brain of mixed glial and mesenchymal cell population. Dr. Swenberg diagnosed this animal as having a small astrocytoma present in the ventral portion of one hemisphere of the forebrain. The final tabulation of Dr. Swenberg is below:

At our request the task force sectioned all spinal cord slides (originally only 10/group were sectioned) and didn't find anything

Astrocytoma Incidence as diagnosed by Dr. Swenberg

Males Dose	0 mg/kg	1	5	15	45
animal:	B23025			B23376 B23377	B23473 B23476 B23479 B23492 B23500 B23505
Females		B23185	B23302	B23442	B23546

these were single tumors only - no animal was double counted.

24-month mouse oncogenicity study:

Performing laboratory: Hazleton Laboratories, Vienna, Va.

Date: 1/15/87(?)

2,4-D (97.5% purity) was administered to 60 $B_6C_3F_1$ Crl Br mice/sex/group for 24 months with an interim sacrifice at 52 weeks of 10/sex/group. Doses administered were 0, 1, 15 and 45 mg/kg/day, administered in the diet. Body weight, food consumption and physical exam results were recorded weekly through week 14 and biweekly thereafter. Blood for hematology was collected from 10/sex/group after 52 weeks and at 104 weeks. Clinical chemistry, and urinalysis were not performed. Liver, heart, kidney, testes, ovaries, brain, adrenals and thyroids were weighed at the 52-week interim sacrifice ands at the 104-week terminal sacrifice.

There was no treatment-related increase in mortality. The number of males that died on test or were sacrificed in extremis prior to terminal sacrifice were 10, 6, 10 and 11 for groups 1-4 respectively. Numbers of females that died on test were 12, 8, 17, and 15 for groups 1-4 respectively. There were no treatment-related effects on body weight or body weight gain, food consumption, hematology or gross pathology. At 52 weeks that was an increase in absolute combined kidney weights in group 4 females, and an increase in absolute and relative adrenal weights in males at all doses tested. At 104 weeks, combined female relative and absolute kidney weights were increased in group 4 and kidney/body weight ratios were also increased in group 3 females and group 4 males. Absolute and relative adrenal weights in males were elevated in group 3 and 4 males at 104 weeks. There were no associated histopathological changes in the adrenals at 52 and 104 weeks accompanying the weight increases. There was, however, an increase in cytoplasmic homogeneity of the renal tubular epithelium due to a reduction of cytoplasmic vacuoles in the mid and high dose kidneys in males at 52 and 104 weeks and in unscheduled deaths. Low dose males were also effected in the unscheduled death group. However, when these animals are combined with the scheduled deaths, no significance was noted. No treatment-related increased incidence in tumors was seen at 52 weeks, 104 weeks or in unscheduled deaths.

target organs kidney + adrenal -

NOEL = 1mg/kg.

Jerry Blondell

IX. Epidemiology study:

The study of interest is "Agricultural Herbicide use and risk of Lymphoma and soft tissue sarcoma. S. Hoar, A. Blair, F. Holms et al. J.A.M.A., 256, 1141-1147, 1986. This was the report of a population-based case-control study conducted by the National Cancer Institute in Kansas. The report stated that they found an association between farm herbicide use (phenoxyacetic acids) and non-hodgkins lymphoma (NHL) but did not find an association with soft tissue sarcoma (STS) or Hodgkin's disease(HD). Four reviewers were asked to evaluate this study and the results of their deliberations are in appendix F. Jerome Blondell, Health Statistician of EPA has summarized the key points from the Peer Review comments and these points are listed below and appended in appendix F.

1. The NCI study by Hoar et al. was well-designed, competently conducted and carefully analyzed.

2. Half of the respondents were next-of-kin who are unlikely to remember accurately which herbicides were used over 10 to 20 years ago.

3. Inconsistencies between subjects' reported use of herbicides and reported use in USDA and EPA surveys in the Kansas area suggest a very serious potential for inaccurate reporting of exposure.

4. Statistically significant findings, particularly those implicating 2,4-D, were based on a very small number of cases, usually 10 or less. Moderate amounts of exposure misclassification, described above, might easily make these findings nonsignificant.

5. The study found a significant association between non-Hodgkins lymphoma and fungicides even with or without exposure to herbicides. The study also found that five groups of herbicides exhibited higher odds ratios for NHL than did phenoxyacetic acids. These two findings cast serious doubt on the specificity of 2,4-D/NHL association.

6. Occupation was not controlled for in the analysis. Those who live on farms have lifestyles, diets, physical activity and exposure to viruses that greatly differ from the general population. These factors are known to affect many kinds of cancer and, as a result, are a potential source of confounding.

7. Reviewers had conflicting views on the strength of support from other studies. The Sweedish studies by Hardell which seem to provide the strongest case for a chlorophenoxy herbicide/NHL association were characterized as having "important methodologic limitations" even by the most favorable reviewer.

8. One of the external reviewers felt that serious regulatory action to limit exposure to 2,4-D should be considered. Two other reviewers did not feel that the weight of evidence implicates 2,4-D as a cause of NHL.

9. The EPA Guidelines for Carcinogen Risk Assessment do not distinguish well enough between the categories "limited evidence of carcinogenicity" and "inadequate evidence" to say with certainty which category the NCI study belongs to.

Reviewed by: David G Anderson David G Anderson 10/23/86
Section VII, Tox. Branch (TS-769C)
Secondary reviewer: Albin B Kocialski
Section VII, Tox. Branch (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: Addendum to the study of 2,4-D on Two-Generations of Reproduction in Rats: Correction to histopathology of the kidneys.

TEST SUBSTANCE: 2,4-Dichlorophenoxyacetic Acid (2,4-D)

SYNONYMS: 2,4-D TOX. CHEM. NO. 315

ACCESSION NO.: 265489.

SPONSOR: Industry Task Force on 2,4-D Research Data (ITF)

TESTING FACILITY: Wil Research Laboratories, Inc. (WIL)
Ashland, OH 44805-9281

TITLE OF REPORT: A Dietary Two-Generation Reproduction Study in Fischer 344 Rats with 2,4-Dichlorophenoxy-acetic Acid: Addendum to the final report.

AUTHORS: Dean E Rodwell, and W. Ray Brown.

STUDY NO.: WIL-81137, same study no. as accession number 265489.

TESTING PERIOD: November 16, 1982 to May 15, 1984.

REPORT ISSUED: September 30, 1986.

PURITY OF TEST SUBSTANCE: See original review of accession no. 259442-6.

CORE GRADE: Not applicable.

CONCLUSIONS ON THE EFFECT AND NO EFFECT LEVELS:

The effect levels for the F0 and F1 males were altered but the no effect levels described in the review of the original study are not altered by the results submitted in this addendum. The LEL and NOEL are restated on the following page. They include kidney histopathological findings reported in this addendum.

LEL and NOEL is expressed in mg/kg/day(Nominal dose level in mg/kg/day).

F0 parental toxicity
LEL- 19.9(20), degeneration of male kidney tubules.
NOEL- 5(5)
F1 parental toxicity
LEL- 14(20), kidney histopathology in males, and reduced body weight in females.
NOEL- 3.8(5)
Developmental toxicity
LEL- 26(20), F1b pup weight reduction.
NOEL- 7.2(5)

Target or nominal dose levels administered in the study are 0, 5, 20, or 80 mg/kg/day.

CONCLUSIONS:

The reexamination of the kidneys from the 2-generation study on reproduction indicated tubule degeneration in males of the F0 and F1 generations which apparently had not yet developed in 28 day old pups. Cortical tubule degeneration(observed mostly in the proximal convoluted tubules) was confined to the F0 males nominally dosed at 80 mg/kg/day, probably because no F1 animals were dosed at this level passed weaning. Most of these pups died prior to weaning; thus, the study was not continued past weaning. No test substance related kidney histopathology was observed in the remaining pups at any dose level. Both the F0 and the F1 male generations nominally dosed at 20 mg/kg/day demonstrated minimal degeneration of tubules in the outer medullary region of the kidney, but not in the cortical region. No test substance related effects occurred at the nominal dose level of 5 mg/kg/day.

These kidney findings on reexamination cast doubt on the quality of the histological examination conducted in the study on reproduction previously reviewed.

A. MATERIALS AND METHODS:

Kidney sections prepared on male rats from the F0 and F1 generations and the F1b pups dosed in the two-generation study of the effects of 2,4-D on reproduction in rats were reexamined.

Target or nominal dose levels(mg/kg/day)	Number of rats reexamined (Tissue sections from these animals) F0	F1	F1b
0 (Control)	30	29	10
5 (LDT)	29	30	10
20 (MDT)	30	29	10
80 (HDT)	30	0	14

F0 males were dosed approximately 40 weeks prior to sacrifice. F1 males were dosed approximately 47 weeks, including 3 weeks in utero and 4 weeks of lactation form the milk and from the mothers food supply especially during the last half of the lactaction period. F1b pups(from which the F1 generation was formed) were dosed for 7 weeks as indicated above, 3 weeks in utero and 4 weeks of lactation. The HDT F1 generation males were not reexamined because of poor survival at this dose level.

B. RESULTS:

The results of the histological reevaluation of the male kidneys are presented in Table 1. Tubules of outer medullary region were characterized in the report as demonstrating probable degenerative or atrophic changes of the epithelial cells in the mid and high dose groups. The involved segments were small and the appearance of increased nuclear density was the result of condensation of the effected portions of the tubule.

In addition to the medullary involvement the cortical tubules (mostly the proximal convoluted tubules) of the high dose F0 generation were large and demonstrated a dense, eosinophilic cytoplasm. The lumens of some of these tubules were indistinct when compared to controls. No F1 adult animals were studied at the highest dose level because of death due to excessive toxicity.

In the mid dose, the histology of the kidneys tubules from F0 and F1 males was less clear, but 7/30 F0 animals were reported to demonstrated the increased nuclear density and 4/29 F1 male rats demonstrated similar histopathology(Table 1).

Other sporatic effects occurred in the kidneys with no apparent dose related response.

C. DISCUSSION:

The kidneys of the F0 male rats from the high dose group in the study of reproduction demonsrated degenerative changes in the tubules of the cortical region and the outer medullary region. In the mid dose groups of the F0 generation and the F1 generation (the highest dose level studied in the F1 generation), less distinct changes occurred and they occurred only in the medullary tubules. No dose related kidney effects were seen in the F1b pups at any dose level.

Thus, no effect level for the study on reproduction did not change, however the effect level in adult rats must now include kidney histopathology and reduced weight gain. Prior to the addendum, the lowest effect level was characterized by only a reduced weight gain in adults and pups.

NOTE:

Since this reexamination of the kidney histology was conducted only after the sponsor identified these effects in a rat subchronic study, there is doubt about the quality of the original histological examination conducted in the reproduction study. The reexamination was conducted by Ray Brown of Research Pathology Associates but the original histological examination was conducted by the testing facility, Wil Research Laboratories. Other organs examined histologically by Wil Research but not by Ray Brown are the epidydimis testis, uterus, and ovary. The study on reproduction gave no indication that the kidneys nor any other organ required histological examination.

Table 1.

Incidence of findings reexamination of kidneys from males
from a 2-generation study of the effects of 2,4-D on reproduction.

Adults or pups	F0 adults				F1 adults			F1b pups			
Nominal dose group:	1	2	3	4	1	2	3	1	2	3	4
Number of rats/group:	30	29	30	30	29	30	29	10	10	10	14
Number examined:	30	29	30	30	29	30	29	10	10	10	14
Number normal:	18	21	16	1	12	16	12	9	9	9	11
Description:											
Increased cytoplasmic eosinophilia in cortical tubules.	0	0	0	28	0	0	4	0	0	0	0
Increased focal nuclear density in medullary tubules.											
minimal	0	0	7	17	0	0	4	0	0	0	0
slight	0	0	0	4	0	0	0	0	0	0	0
moderate	0	0	0	1	0	0	0	0	0	0	0
Total incidence	0	0	7	22	0	0	4	0	0	0	0
Multifocal tubular degeneration/basophilia											
minimal	10	8	8	3	13	8	10	1	0	1	3
slight	0	0	0	0	0	2	0	0	0	0	0
Total incidence	10	8	8	3	13	10	10	1	0	1	3
Microcalculi	1	1	1	1	2	4	1	0	1	0	0
Pelvic dilation/hydronephrosis, unilateral.	1	0	1	0	2	1	1	0	0	0	0
Focal/multifocal mononuclear cellular infiltation.	1	1	0	0	3	1	0	0	0	0	0
Focal tubular dilation.	0	0	0	0	3	2	6	0	0	0	0
Focal/multifocal chronic nephritis.	0	0	0	0	1	0	1	0	0	0	0
Pelvic mineralization	0	0	0	0	0	1	0	0	0	0	0
Focal papilllary edema	0	0	0	0	0	0	1	0	0	0	0

Nominal dose groups: 1 = 0 mg/kg/day, 2 = 5 mg/kg/day, 3 = 20 mg/kg/day, 4 = 80 mg/kg/day.

The following organs and tissues were taken at sacrifice and preserved, but histopathology was conducted only as previously indicated.

1. Adipose tissue
2. Adrenals
3. Aorta
4. Bladder
5. Bone marrow
6. Brain
7. Cecum, colon
8. Spinal cord
9. Epididymis
10. Esophagus
11. Eyes
12. Ovaries/Testes
13. Heart
14. Intestines
15. Kidneys
16. Liver
17. Lung/bronchi
18. Lymph node-thoracic and mesenteric
19. Mammary Gland and Skin
20. Nasal turbinates
21. Pancreas
22. Parathyroids
23. Pituitary
24. Prostate
25. Salivary Glands
26. Sciatic Nerve
27. Seminal Vesicle
28. Skeletal Muscle
29. Spinal Cord
30. Spleen
31. Sterum
32. Stomach
33. Thymus
34. Thyroid
35. Trachea
36. Uterus/cervix
37. Vagina

Statistical Methods

All analyses were conducted using two-tailed tests (unless otherwise specified).

1. Histopathological findings and incidence by sex were compared to control groups by Kalmogorov-Smirnov one-tailed test.

2. F0 and F1 male and female fertility indexes, F1a, F1b, F2a, and F2b pup sex ratios on lactation day 1, and F1a, F1b, F2a, and F2b pup survival indexes on on lactation day 4, 7, 14, 21, and 28 for the control groups were compared to each treated group by the Chi-square test with Yates correction factor.

3. Other effects in treated groups were compared to controls by analysis of variance followed by Dunnett's test.

Summary of Study Conduct

1. Test substance administered continuously throughout all phases of the study.

2. F0 dosed continuously from approx. 5 to 6 weeks of age for 105 days prior to first mating (i.e., approx. 20 weeks of age).

3. F0 mated 1:1 for 10 days and if no evidence of sperm, second matings were allowed with a proven male for 5 days.

4. F0 continued for 3 weeks of gestation and 4 weeks to weaning of F1a litters. Pups reduced to 8 per dam on day 4 of lactation.

5. All F1a litters necropsied and discarded after weaning.

6. F0 rested 2 weeks between weaning F1a and mating for production of the F1b as in #3.

7. F0 continued for 3 weeks gestation and 4 weeks to weaning of F1b litters. Pups reduced to 8 per dam on day 4 of lactation.

8. Ten F1b pups per sex per dose level randomly selected for necropsy, after weaning.

9. One pup per sex per dam per dose level randomly selected from F1b litters for the F1 generation. Because of excess toxicity at the target dose level of 80 mg/kg, only controls, and the target dose level groups of 5 and 20 mg/kg were continued on study. All F1b pups at 80 mg/kg/day were sacrified at the end of weaning.

10. All F0 animals were sacrificed on week 40 of the study.

11. Selected F1 pups were dosed via milk and in the feed for 125 days prior to mating to produce the F2a litters.

12. Dosing, mating, gestation, and weaning in the F1 generation producing the F2a and F2b litters followed procedures, including necropsy, similar to those followed for the F0 generation in producing the F1a and F1b litters.

13. All F1 animals were sacrificed on week 77 of the study.

14. All F1a, F1b, F2a, and F2b dying prior to weaning were studied for malformations and variations.

D. Test Chemical Identity and Concentration in the Feed

The study report, presented an analysis conducted by Wil Research Laboratories, and the Industry Task Force analysis on 2,4-D. According to a Wil Research analysis, the test substance was 95.8 percent pure 2,4-D. The report presented the following analysis of the test substance by the task force, but no further analysis or explanation of the differences between the Task Force analysis of 97.5 percent, and the Wil Research analysis of 95.8 percent, was presented.

[redacted]

Samples of the diets containing 2,4-D were collected for study weeks 0, 1, 2, 3, 4, 8, 13, 26, 39, 52, 65, 77. None of the sample diets were collected during weeks of gestation or lactation. The analyses after recovery of 2,4-D from the diets with the highest concentration were within 10 percent of the measured concentration. Analyses of 2,4-D in the diets at the middle dose level and the lowest dose level were always within 15 percent to 20 percent of the measured concentrations, except for three of the lowest dose levels which were 77 percent, 61 percent, and 55 percent of the measured dose levels. One was in a diet mixed on the 4th week of the study and two were for a diet mixed on the 13th week of the study. The 55 percent of the measured level was apparently a repeat analysis on a sample of the diet yielding the 61 percent of measured dose level.

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E. Results

1. Fertility in F0 and F1 Males and Females.

No reduced fertility was expressed in males or females of the F0 generation in producing either the F1a or the F1b litters. However, a nonstatistically significant apparent reduction in male fertility occurred in producing the F1b litters (table 3). No reduced fertility was expressed in males or females of the F1 generation in producing the F2a and F2b litters. A second mating by a proven male was conducted when females demonstrated no evidence of sperm. The number of second matings producing the F1a/F1b pups were 0/6, 5/6, 1/2 and 0/2 for controls and the target dose levels of 5, 20, or 80 mg/kg/day, respectively. Second matings to produce F2a/F2b pups were 3/4, 2/1, and 4/4 for control and the target dose levels of 5, or 20 mg/kg/day.

The fertility index for production of the F1a and F1b litters is 70 to 79 percent in control F0 males and 70 to 79 percent in control F0 females (see table 3). The fertility index for males and females, respectively is the number of gravid females divided by the number males or females mated, respectively, adjusted to percent. These indexes ranged from 70 to 83 percent in treated males and 70 to 90 percent in treated females producing the F1a and F1b litters. Similarly, the fertility index for production of F2a and F2b litters is 60 to 70 percent in control F1 males and 64 to 72% in control F1 females (table 4.). These indexes range from 67 to 80 percent in treated F1 males and 64 to 80 percent in treated F1 females producing F2a and F2b litters. None were statistically significantly different from controls. The number of days required for mating ranged from 4.0 to 5.7 days of cohabitation to produce the F1a and F1b litters and 3.2 to 4.6 days of cohabitation to produce the F2a and F2b litters. These were no different from control values.

This failure to detect an effect on fertility is consistent with the lack of histopathological findings in the testes or epididymides of males and with the lack of histopathological findings in the ovaries or uteri of females from the F0 or F1 generation at terminal sacrifice. However, since the highest dose level was dropped from the study, fertility in the F1 generation was not evaluated at this dose level. Thus, the mid target dose level of 20 mg/kg/day should be considered the NOEL for fertility.

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Table 3

Fertility indexes for F0 male and females producing F1a and F1b litters.

Fertility Index (no. gravid/no. males or females mated) x 100

	Producing F1a				Producing F1b			
Target dose	No. of males	%	No. of females	%	No. of males	%	No. of females	%
0	21/30	70	21/30	70	23/29	79	23/29	79
5	25/30	83	26/30	87	25/30	83	27/30	90
20	24/30	80	24/30	80	23/30	77	23/30	77
80	21/30	70	21/30	70	21/30	70	21/30	70

Table 4

Fertility indexes for F1 male and females producing F2a and F2b litters.

Fertility Index (no. gravid/no. males or females mated) x 100

	Producing F2a				Producing F2b			
Target dose	No. of males	%	No. of females	%	No. of males	%	No. of females	%
0	21/30	70	21/30	72	18/30	60	18/28	64
5	24/30	80	24/30	80	20/30	67	20/30	67
20	22/30	73	23/30	77	20/30	67	20/30	67

2. Length of Gestation in F0 and F1 Females

The lengths of gestation was statistically significantly prolonged in F0 females producing the F1b pups only and only at the highest target dose level of 80 mg/kg/day. This increase in gestational lengths was due to a gestation length of 23 days in approximately one half of the dams from this group instead of the usual 22 days of gestation demonstrated by most F0 and F1 dams in all groups. The LEL is between 103 and 114 mg/kg/day and NOEL is between 18 and 35 mg/kg/day.

The effect could result from delayed implantation, hormonal imbalance, or parturition problems. The effect is considered biologically significant and undesirable.

3. Body Weights of the F0 and F1 Generations.

The mean body weights of F0 males and female rats were statistically significantly less than controls in the high dose group only. In F0 males, the reduced body weight (97 percent of controls) was consistent after the sixth week of test substance consumption and in F0 females the body weight was consistently reduced (96 percent of controls) by the twelfth week of test substance consumption. The failure to gain as much weight as controls could not be attributed to reduced food consumption. The food consumption, and the food consumption per gram body weight gain was slightly increased. Body weights of the F0 generation in the target dose groups of 5 or 20 mg/kg were similar to control weights throughout this study, but food consumption appeared to be slightly elevated(not satistically significant).

F0 dams producing F1a and F1b litters had statistically significantly lower body weights than control weights on day 20 of the gestation producing the F1a and F1b litters in the highest dose group (table 5). At this dose level, body weights of dams were reduced on day 7, 13, and 20 of the gestation producing F1a litters, but the body weights of dams producing F1b litters were statistically significantly reduced only on day 20. Thus, toxicity was expressed in F0 dams during gestation of the F1a and F1b litters.

On lactation day 7, F0 dams lactating for F1a litters, express significantly reduced body weights in the highest dose group (table 5). For these dams, the body weight per gram of food consumed was about one half the value when compared to other dose groups and controls(data not shown). Dams demonstrated toxicity during lactation for the F1a, and for the F1b litters. At the end of lactation for the F1a and F1b litters, the body weights were satistically significantly elevated.

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Table 5

F0 Female Body Weight (g) during gestation and lactation for F1a and F1b litters.

	Target Dose Levels (mg/kg/day)			
	0	5	20	80
	Body wt. of F0 during gestation producing F1a			
Day 0	178	179	178	173
7	190	191	191	181**
13	208	208	206	196**
20	246	252	249	232*
	Body wt. of F0 during lactation for F1a			
Day 0	189	191	191	184
7	205	207	201	189**
14	212	212	207	208
21	216	213	219	212
28	189	184	185	204**
	Body wt. of F0 during gestation producing F1b			
Day 0	200	205	202	197
7	210	214	210	204
13	226	232	230	218
20	270	277	274	244**
	Body wt. of F0 during lactation for F1b			
Day 0	210	215	208	205
7	226	233	225	211*
14	228	237	233	224
21	229	239	234	231
28	203	197	193	226*

*p < 0.005, Dunnett's Test.
**p < 0.01, Dunnett's Test.

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Table 6

F1 Female Body Weight (g) during gestation and lactation for F2a and F2b litters.

	Target Dose Levels (mg/kg/day)		
	0	5	20
	F1 during the gestation producing F2a		
Day 0	201	198	198
7	211	208	211
13	234	227	228
20	271	271	270
	F1 during lactation for F2a		
Day 0	216	211	211
7	228	221	222
14	234	232	233
21	232	233	236
28	220	221	223
	F1 during gestation producing F2b		
Day 0	222	221	214*
7	234	229	224*
13	250	248	241
20	293	290	278
	F1 during lactation for F2b		
Day 0	236	234	227*
7	248	245	237
14	260	245*	248
21	255	252	250
28	228	221	222

*$p < 0.05$, Dunnett's Test.

The body weights of the F1 generation, after selection, was comparable to control body weights, except in females at the target dose level of 20 mg/kg during weeks 74 to 77 where they were statistically significantly less than controls (97 percent of controls). The report stated that these body weight reductions in females were not biologically significant. No explanation was presented.

The body weights of F1 females during gestation and lactation demonstrated no consistently significant patterns during production or lactation for the F2a or F2b litters (table 6), however they were statistically significantly reduced on day 0 and 7 of the gestation producing the F2b litters, and on day 0 of lactation for the the F2b litters.

4. Pup Weights from F1a, F1b, F2a, and F2b Litters

Pup weights were significantly reduced over control weights in the F1a (table 7) and F1b (table 8) pups only. Both male and female F1a and F1b pup weights were less than control weights from birth to lactation day 28 in the 80 mg/kg target dose group. At the next lower dose level, both F1a and F1b male and female pup weights tended to be apparently lower than control weights toward the end of lactation. By day 20 of lactation, both male and female pups in the F1b litters only demonstrated a statistically significant decrease in body weight over control weights. The male pup weight in F1b litters in the lowest dose group which were statistically significantly reduced on lactation day 28 may not be biologically significant, since there were no apparent differences from control weights throughout the previous weeks of lactation.

None of the F2a or F2b pup weights were found to be different from control weights.

Table 7

Summary of Fla litter weights (g)
males and females

Group No.	Dose Level (mg/kg/day)	Males Mean S.D.	Lactation Days 1	4 Before Selection	4 After Selection	7	14	21	28
1	0	Mean	5.5	7.7	8.0	11.9	22.5	32.6	51.8
		S.D.	0.83	1.50	1.06	1.15	1.72	2.81	6.33
2	5	Mean	5.6	7.9	7.9	11.8	22.1	31.7	48.8
		S.D.	0.71	1.20	1.22	1.83	2.67	3.06	6.37
3	20	Mean	5.6	7.9	7.9	11.8	21.3	30.9	48.0
		S.D.	0.61	0.71	0.71	0.80	2.26	2.59	5.62
4	80	Mean	4.9*	6.4**	6.4**	8.5**	17.2**	26.7**	39.1**
		S.D.	0.46	0.71	7.22	1.30	2.10	2.22	5.24
Group No.		Females							
1	0	Mean	5.2	7.5	7.7	11.4	21.7	31.1	48.8
		S.D.	0.72	1.41	0.94	1.03	1.91	2.87	5.25
2	5	Mean	5.4	7.7	7.7	11.5	21.5	30.5	46.0
		S.D.	0.73	1.24	1.25	1.76	2.55	2.74	5.47
3	20	Mean	5.4	7.7	7.7	11.5	20.7	30.0	46.0
		S.D.	0.75	0.58	0.59	0.66	2.27	2.79	5.28
4	80	Mean	4.7	6.3**	6.3**	8.5**	17.0**	26.5**	39.3**
		S.D.	0.39	0.85	0.85	1.46	2.57	3.10	6.30

* = Significantly different from control group at .05 level using Dunnett's test.

** = Significantly different from control group at .01 level using Dunnett's test.

Table 8

Summary of Flb litter weights (g)
Males and females on
lactation days

Group No.	Dose Level (mg/kg/day)	Males Mean S.D.	1	4 Before Selection	4 After Selection	7	14	21	28
1	0	Mean	5.8	8.5	8.5	12.4	23.9	34.6	56.0
		S.D.	0.43	0.77	0.77	1.17	2.31	3.58	8.87
2	5	Mean	5.6	8.4	8.4	12.5	23.9	34.3	50.6*
		S.D.	0.58	0.92	0.92	1.32	2.19	3.34	5.17
3	20	Mean	5.4	7.9*	7.9*	11.8	22.7	32.6	47.2**
		S.D.	0.50	0.63	0.63	0.86	1.18	2.34	7.26
4	80	Mean	4.5**	5.2**	5.2**	7.2**	15.9**	26.3**	41.1**
		S.D.	0.44	1.14	1.16	1.80	3.57	4.23	6.58
Group No.		Females							
1	0	Mean	5.3	8.1	8.1	11.7	22.5	32.3	51.0
		S.D.	0.46	0.83	0.83	1.11	1.89	2.95	7.52
2	5	Mean	5.3	8.0	8.0	11.9	22.9	32.6	47.4
		S.D.	0.59	0.83	0.84	1.19	1.79	2.59	4.56
3	20	Mean	5.2	7.6	7.6	11.2	21.7	30.9	44.2**
		S.D.	0.46	0.57	0.62	0.68	1.01	2.06	6.77
4	80	Mean	4.4**	5.6**	5.5**	7.2**	15.1**	25.0**	39.0**
		S.D.	0.54	0.83	0.80	1.23	1.00	0.91	1.47

* = Significantly different from control group at .05 level using Dunnett's test.
** = Significantly different from control group at .01 level using Dunnett's test.

The reduced F1b pup body weights in the mid dose level occurred from lactating dams demonstrating no statistically significant toxic signs at the time, although their body weights were apparently reduced from controls on lactation day 28. This may indicate that a change in the metabolism of 2,4-D occurred in F0 dams from production of the F1a to production of the F1b litters. Thus, dams exhibiting apparently no toxicity at the time, resulted in a reduction in pup weight over control weights.

5. Viability of F1a, F1b, F2a, and F2b Litters

The study demonstrated a statistically significantly reduced pup viability over controls only at the highest target dose level of 80 mg/kg (tables 9 and 10). The greatest reduction occurred in F1b pups at birth, with the mean litter size being about one half the control value due to deaths of portions and of entire litters. The mean litter size was reduced from five to three by day 14 of lactation, with no more deaths by lactation day 28 (table 10).

Some indication of reduced litter size was apparent in F1a litters of the target dose of 80 mg/kg, but the apparent decrease was not statistically significant (table 9). At birth however, there was a difference in the sex ratio of pups which was significant at the $p < 0.01$ level. From day 1 to day 28 of lactation, no further significant number of pup deaths occurred.

The study report stated that the decrease in female pups at births in F1a litters was not dose-related. I believe that it may be dose related, since at the highest test substance consumed by mothers producing F1b pups, where test substance consumption was higher than in dams producing the F1a litters, both male and female pup survival at birth were less in these F1b pups than the corresponding pup survival in the F1a pups. Thus, there appeared to be a dose response relationship.

Viability of the F2a and F2b pups was not affected.

6. Malformations and Variations

F1b pups which died before lactation day 28 were studied for malformation and variation. As can be seen from table 11, bent ribs, 14 the rudimentary ribs, malaligned sternebrae and unossified sternebrae were seen in the F1b pups. Since most of these pups died at birth or were dead by day 1 of lactation, the effects were seen primarily just after birth at the highest dose only and in the F1b pups only. This was the only group for which there were sufficient deaths, and animals could be necropsied. Only pups which died were available for necropsy except at weaning. These effects are

Table 9
Summary of Fla viability indexes

Group No.	No. Dead Pups	Sex Ratios Day 1 M:F	Live Litter Size No.	Live Litter Size MEAN	Gestation Survival Index No.	Gestation Survival Index %	Day 4 Before Selection No.	Day 4 Before Selection %	Day 4 After Selection No.	Day 4 After Selection %
1	3	99:114	213/21	10.1	213/216	98.6	208/213	97.7	160/160	100.0
2	20	133:118	251/25	10.1	251/271**	92.6	247/251	98.4	191/191	100.0
3	3	121:116	237/24	9.9	237/240	98.8	237/237	100.0	183/183	100.0
4	9	109:71**	180/20	9.0	180/189	95.2	175/180	97.2	147/147	100.0

Group No.	Day 7 No.	Day 7 %	Day 14 No.	Day 14 %	Day 21 No.	Day 21 %	Day 28 No.	Day 28 %
1	156/160	97.5	156/160	97.5	156/160	97.5	156/160	97.5
2	190/191	99.5	190/191	99.5	190/191	99.5	190/191	99.5
3	183/183	100.0	183/183	100.0	183/183	100.0	183/183	100.0
4	146/147	99.3	143/147	97.3	143/147	97.3	143/147	97.3

1 - 0 mg/kg/day 2 - 5 mg/kg/day 3 - 20 mg/kg/day 4 - 80 mg/kg/day

Survival ratios and sex ratios compared using chi-square test.
Mean number of viable pups compared using analysis of variance.
** = Significantly different form control at .01 level.
Live litter size = No. pup alive on day 1 of lactation/no. litters.
Gestation index = No. pups alive on day 1 of lactation/total no. pups born.
Viability indexes = No. pups alive on day 4 before selection/no. pups alive day 1.
= No. pups alive day n/no. pups alive day 4 after selection.

Table 10
Summary of F1b viability indexes

Group No.	No. Dead Pups	Sex Ratios Day 1 M:F	Live Litter Size No.	Live Litter Size MEAN	Gestation Survival Index No.	Gestation Survival Index %	Day 4 Before Selection No.	Day 4 Before Selection %	Day 4 After Selection No.	Day 4 After Selection %
1	5	112:107	219/23	9.5	219/224	97.8	219/219	100.0	164/164	100.0
2	15	120:131	251/25	10.0	251/266	94.4	246/251	98.0	177/177	100.0
3	3	110:128	238/23	10.4	238/241	98.8	237/238	99.6	174/174	100.0
4	110**	23:28	180/20	5.1**	51/161**	31.7	44/51**	86.3	42/42	100.0

Group No.	Day 7 No.	Day 7 %	Day 14 No.	Day 14 %	Day 21 No.	Day 21 %	Day 28 No.	Day 28 %
1	164/164	100.0	164/164	100.0	164/164	100.0	164/164	100.0
2	177/177	100.0	177/177	100.0	176/177	99.4	176/177	99.4
3	174/174	100.0	174/174	100.0	174/174	100.0	174/174	100.0
4	34/42**	81.0	30/42**	71.4	30/42**	71.4	30/42**	71.4

1 - 0 mg/kg/day 2 - 5 mg/kg/day 3 - 20 mg/kg/day 4 - 80 mg/kg/day

Survival and sex ratios compared using chi-square test.
Mean number of viable pups compared using analysis of variance.
** = Significantly different from control at .01 level.
Live litter size , gestation index and viability indexes = see legend table 9.

sometimes seen at dose levels causing maternal toxicity, but administration of many compounds do not cause these effects at maternally toxic dose levels.

The number of malformations and variations in these F1b pup dying prior to weaning were apparently not sufficient for statistical significance by the Fischer exact test. As can be seen from Table 11, 50 percent of the litters which died in the high dose group had, for example, malaligned sternebrae compared with 20 percent in controls. The adequacy of these statistical evaluations appear questionable and perhaps should be reevaluated by OPP. However, even if the number of anomalies and variations were significant in the high dose group, the failure to find significant numbers of these effects in five litters examined in each of the controls and the lowest dose group may indicate that these effects did not occur below the highest dose level.

If comparable examinations were conducted in all F1a pups, a dose relationship may have been apparent in the anomalies and variations. There is no indication that this was done. A detailed study on developmental effects on the F1a pups which died during lactation was conducted but these numbers were insufficient to establish a NOEL. If the F1a pups were preserved, it may have been useful to have examined them for a dose related response in developmental effects. However, by day 28 of lactation, all of the apparent effects anologus to those seen in the F1b pups shortly after birth may have disappeared.

Dose levels consumed by dams around the perinatal period were greater for the F1b litters than for the F1a litters. The week immediately before parturition, gestational day 13-20, the dams of the F1a pups consumed the test substance at a daily rate of 76.1 mg/kg, while the dams of the F1b pup, during the corresponding time period consumed 107 mg/kg. The daily consumption of test substance by dams during the first week of lactation for the F1a and F1b pups was 112 and 133 mg/kg, respectively, in the 80 mg/kg target dose level group.

Table 11.

Total Number of Pups and Litters with Developmental and Genetic Variations - Only F1b Pups Found Dead Lactation Days 0-28

Dose Group	Pups 1	2	3	4	Litters 1	2	3	4
Number Examined Externally	5	15	3	103	5	5	3	18
Findings	----None----------				----None----------			
Number Examined Viscerally	5	15	3	103	5	5	3	18
Findings	----None----------				----None----------			
Number Examined Skeletally	5	14	3	98	5	5	3	18
Sternebra #5 and/or #6 Unossified	0	0	0	7	0	0	0	5
Sternebrae #1, #2, #3 and/or #4 Unossified	0	0	0	1	0	0	0	1
Sternebrae Malaligned (slight or moderate)	1	0	0	23	1	0	0	9
14th Rudimentary Rib(s)	0	0	0	12	0	0	0	6
Bent Rib(s)	0	0	0	30	0	0	0	6
Reduced Ossification of the Vertebral Arches	0	0	0	2	0	0	0	2

None significantly different from control group using Fisher's Exact Test.

7. Organ Weights and Histological Studies

The absolute and relative liver weights were reduced at all dose levels in the F0 males (table 12). Only the liver/body weight ratios are presented. The absolute and relative kidney weights were increased at all dose levels in F0 females (table 12). The report did not consider the effects on organ weights to be dose-related in either sex. No explanation for this opinion or for these possible test substance related effects was presented. However, neither effect exhibited a smooth dose-related decrease or increase, respectively.

In the F1 generation relative kidney weight of the left but not the right kidney was significantly elevated in males at the 20 mg/kg target dose level only (table 13). The relative liver weights in males of this group were apparently elevated but not statistically. The relative liver weights were increased in F1 females of this dose group but the apparently slightly elevated kidney weights, probably, are not dose related (table 13). Thus, the possible oragn weight effects in F1 generation failed to confirm the statistically significant organ weight effects seen in the F0 generation.

No organ weight effects or histopathology was seen in the testes from any dose level from any generation. No dose-related histological effects were seen in the ovary. Thyroids may have been saved but no histology was conducted on them. All the histological studies conducted failed to find any dose-related pathology in any of these organs in the F0 generation and the F1a, F1b, and F1 generation and F2a and F2b pups.

Two histological studies on the livers of the F0 animals were reported. One study was conducted by the testing facility (table 14), and the other was conducted by W. Ray Brown of Research Pathology Services,Inc., New Britain, P.A.(table 15).

When the livers from F0 males were examined histologically numbers of small foci of necrosis were found in all groups. This was initially diagnosed as Tyzzer's desease (table 14). This diagnosis was rejected because females were not affected, diarrhea was not detected, and survival was normal. Research Pathology Services found that small basophilic alterations in hepatocytes occurred at a slightly higher incidence in dosed animals(table 15). In females, these alterations occurred at a slightly higher incidence in controls. None of these histological findings were considered to be dose related by either pathologist.

Table 12.

F0 Terminal Body Weights and Relative Organ Weights

	Target Dose Levels mg/kg/day			
	0	5	20	80
F0 male bwt.	372.	373	368	354**
SD	15.4	17.8	18.3	19.5
F0 female bwt.	217	220	216	209**
SD	10.5	9.8	8.4	12.2
F0 male organ wt. per 100 g bwt.				
Lt Kidney	0.417	0.356**	0.421	0.435
SD	0.18	0.06	0.05	0.04
Rt Kidney	0.469	0.357**	0.420	0.429
SD	0.18	0.06	0.05	0.05
Liver	3.474	3.242**	3.337*	3.226**
SD	0.22	0.18	0.17	0.25
Testes	0.830	0.835	0.821	0.854
SD	0.04	0.04	0.05	0.08
F0 female organ wt. per 100 g bwt.				
Lt Kidney	0.351	0.471**	0.410*	0.425**
SD	0.14	0.12	0.05	0.04
Rt Kidney	0.361	0.476**	0.398	0.424
SD	0.15	0.11	0.06	0.04
Liver	3.477	3.663	3.608	3.627
SD	0.21	0.50	0.27	0.20

SD = Standard deviation; * = $p < 0.05$; ** = $p < 0.01$

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Table 13.

F1 Terminal Body Weights and Organ Weight Ratios

	Target Dose Levels[a] mg/kg/day		
	0	5	20
F1 male bwt.	394.	388	386
SD	13.8	28.8	22.9
F1 female bwt.	238	231	231*
SD	9.8	9.0	11.1
F1 male organ wt. per 100 g bwt.			
Lt Kidney	0.394	0.381	0.411*
SD	0.025	0.03	0.02
Rt Kidney	0.390	0.378	0.402
SD	0.02	0.03	0.02
Liver	3.315	3.345	3.439
SD	0.25	0.25	0.17
Testes	0.865	0.857	0.861
SD	0.06	0.11	0.08
F1 female organ wt. per 100 g bwt.			
Lt Kidney	0.398	0.406	0.419
SD	0.03	0.04	0.03
Rt Kidney	0.402	0.400	0.415
SD	0.03	0.05	0.03
Liver	3.568	3.566	3.808**
SD	0.27	0.33	0.25

SD = Standard deviation; * = $p < 0.05$; ** = $p < 0.01$
[a]F1 at 80 mg/kg/day target dose level not dosed beyond weaning.

Table 14.

F0 histomorphological at terminal sacrifice.
Summary incidence for the live.
Testing laboratory summary.

Sex	Male				Female			
Dose group	1	2	3	4	1	2	3	4
Number of animals studied	30	30	30	30	29	29	30	29
Liver								
Total examined	30	30	30	30	29	29	30	29
Examined, unremarkable	6	3	9	13	20	22	20	17
Not examined	0	0	0	0	0	0	0	0
Cholangiofibrosis	21	20	19	14	5	3	5	5
Accessory lobe	1	1	0	3	2	1	0	3
Tyzzer's disease	4	18*	10	1	0	0	0	0
Nonspecific Kupffer cell granuloma	0	0	0	0	4	4	6	5

1= 0 mg/kg/day 2= 5 mg/kg/day 3= 20 mg/kg/day 4= 80 mg/kg/day

* Significantly different from control at 0.05 level, using Kolmogorov-Smirnov, one-tailed test.

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Table 15.

F0 histomorphological summary incidence
for liver, at terminal sacrifice.
Summary from Research Pathology Services

Sex	Male				Female			
Dose group	1	2	3	4	1	2	3	4
Number examined	30	30	30	30	29	29	30	29
Number normal	1	29	23	24	9	7	12	8
Multifocal bile duct proliferation	25	29	23	24	9	7	7	11
Focal necrosis	11	2	1	3	1	1	1	0
Multifocal necrosis	13	19	15	11	0	0	0	0
Focal cellular alteration								
Basophilic-cell focus/foci	0	4	3	6	8	9	3	1
Clear-cell focus/foci	1	0	0	2	0	0	0	0
Eosinophilic-cell focus/foci	0	0	0	0	0	0	1	0
Microgranuloma/s	2	5	4	2	6	3	7	9
Multifocal mononuclear cellular infiltration	6	2	4	3	6	10	6	8
Accessory lobe	1	1	0	3	2	1	0	3
Centrilobular hepato-celular vacuolation	0	0	0	1	0	0	0	0
Focal hepatocellular vacuolation	1	0	0	0	0	0	0	0
Congestion	0	0	0	1	0	0	0	0
Congenital anomaly	0	0	0	0	1	0	0	0

8. Summary and Discussion

1) The study reviewed is a 2-generation, 2 litter per generation study of the effects of 2,4-dichlorophenoxy-acetic acid (2,4-D) on reproduction in Fischer 344 rats.

2) The test substance, (97.5% 2,4-D by an I.T.F analysis; and 95.8% 2,4-D by a WIL analysis) was administered in the feed, ad libitum, to 30 rats per sex per group. The concentration of the test substance was adjusted in the feed weekly or monthly according to food consumption and body weight in an attempt to meet target dose levels of 0, 5, 20 or 80 mg/kg/day. During gestation and lactation the actual dose level administered was generally higher, see table 1 and 2, even with 50 percent reduction in concentration during week 2 and 67 percent reduction in concentration during week 3 and 4 of lactation.

3) No significant effects on fertility of males or females at any dose or in any generation was evident. This conclusion is supported by the failure to find dose related effects on the testes weight or on histological examination of the testes. No dose related histological effects were seen in ovaries. There was no dose related differences in the number of second matings or in the time required for cohabitation. The fertility of Fischer 344 rats is not high, 60-79 percent in controls, and the variability of the fertility probably would allow detection of only severe reductions in fertility.

4) The lengths of gestation was prolonged by 1 day in approximately one half the F0 dams producing F1b litters only in the highest dose group. This effect could result from delayed implantation, hormonal imbalances, or parturition problems.

5) The mean body weights of the F0 generation were statistically significantly reduced compared to controls prior to mating, at the highest dose level. Since body weight gain per gram of food consumed was apparently nearly always less in the high dose group than in the other treatment groups or the controls, the body weight decrease cannot be explained by decreases in food consumption. At this dose level, food consumption was frequently satistically significantly

increased over control values. At the two lowest dose levels, food consumption was generally apparently increased, but it was seldom satistically significant. Thus, the weight reduction probably is real.

6) During lactation, the body weights of F0 dams in the high dose group were not consistently reduced and in the middle dose group in the F0 dams lactating for the F1b litters, there were no statistically significant reductions in body weight compared to controls. Note: It was in the mid dose group and during lactation for F1b litters, that the LEL for pup weight depression occurred.

7) The body weights of F1 females during gestation and lactation for F2a and F2b litters were infrequently significantly different from control weights(see table 6). After weaning of the F2b litters from week 44-77 were adult F1 female body weights significantly less than control weights for the target dose level of 20 mg/kg. The body weights of male F1 rats were not different from control weights at any time after weaning.

8) Pup body weights were significantly reduced over control weights in the F1a and F1b pups only. These reduced pup weights occurred at the highest dose throughout lactation and in the mid dose only toward the end of lactation, and only in the F1b pups. The NOEL was the lowest target dose level administered.

9) Pup viability was reduced at parturition and during the first day of lactation in F1a and F1b pups at the target dose level of 80 mg/kg (actual 76.1 to 133 mg/kg/day) only. A reduction in litter size probably also occurred in the highest dose group in the F1a litters. The apparent reduction probably was dominantly due to a decrease in number of female pups born, causing a significant difference in the sex ratio at birth.

Pup viability was more severely and significantly reduced in the F1b litters than in F1a litters at birth and between birth and lactation day 1 in addition to the period between lactation day 1 and lactation day 4. The sex ratio in these F1b pups was normal, probably because male, in addition to female pup viability, was less than in the F1a litters.

10) Anomalies and variations occurred in F1b litters of the high dose which died during lactation. This was the only group for which those effects could be determined because it was the only group apparently for which skeletal examinations were conducted. In addition, it was the only group in which a large number of nonscheduled pup deaths occurred.

These skeleltal anomalies and reductions in ossification are generally consistent with similar effects produced by 2,4-D in the teratogenicity study in Fischer 344 rats. The NOEL for developmental effects in that study is 25 mg/kg/day.

11) The absolute and relative liver weights of F0 males were statistically significantly reduced at all dose levels at terminal sacrifice. The absolute and relative kidney weights of F0 females were statistically elevated over control weights at all dose levels. There was not a "clean" dose-response relationship and the report did not consider the effect on either sex to be biologically significant.

The liver weight reductions seen in the males may not be toxicologically or pharmacologically significant, and could be an artifact of the study.
a) There was no "smooth" dose response relationship with the liver weight and the dose of the test chemical.
b) F1 males and females demonstrated no liver weight reductions.
c) No significant liver weight reductions occurred in a 90-day subchronic or a chronic study conducted at 1, 5, or 45 mg/kg/day in the Fischer 344 rat.
d) The reductions probably are not due to the slight thyroid effects analogus to the thyroid effects seen in the subchronic and chronic studies, because only higher elevations of T4 than those seen cause glycogen depletion in the liver.

e)The reductions are not due to an interaction of 2,4-D with the liver histological findings seen. The liver weights in control animals with and without focal necrosis, multifocal necrosis, or basophilic alterations were each not different from each other. Similar comparisions failed to detect diffences in the highest dose level group.
f) Food consumption apparently increased at all the higher dose levels, and in some cases the increase was satistically significant. Thus, the liver weight reduction is not due a reduction in food consumption.

The satistically significant kidney weight increase in females of the F0 generation probably are not correlated with the kidney histopathology seen the males and female of the subchronic and chronic studies. No kidney histopathology was seen in any animals in the reproduction study. In addition, the kidney weights of 5 females in control animals were an average of 0.18 g for the left or the right kidney, whereas the average kidney weights in the remaining control animals were 0.9 g for the left or the right kidney, approximately 3 standard deviations different. Thus, if these 5 animals are removed from controls, the kidney weights in dosed animals are comparable to controls.

It is concluded that the kidney weight increase is due to an anomaly in the kidney weights of 5 control females, and that it is not due to the test substance.

12) No significant dose-related histopathology occurred in any organ at any dose level in any generation.

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References:

1. Subchronic toxicity study in Fischer 344 rats conducted by Hazleton Laboratories, Report No. 2184-102, dated September 12, 1983, for the Industry Task Force on 2,4-D Research No. 251474.
Feeding study conducted 90 days at dose levels of 0, 1, 5, 15, or 45 mg/kg/day.

2. Interim 52-week report on 2,4-D chronic feeding/ oncogenicity study in Fischer 344 rats. Conducted by Hazleton Laboratories submitted by the Industry Task Force on 2,4-D Research. Accession No. 256019.

Feeding study conducted at 0, 1, 5, 15, or 45 mg/kg/day.

3. Teratogenicity study of 2,4-D in Fischer 344 rats. Conducted at WIL Research Laboratories (WIL-81135) for the Industry Task Force on 2,4-D Research.

Study conducted at 0, 8, 25, or 75 mg/kg/day by gavage.

4. Reproduction study of 2,4-D in Fischer 344 rats. Conducted by WIL Research Laboratories (WIL-81137) for the Industry Task Force on 2,4-D Research. Accession No's. 259442-6.

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